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BAP (6-Benzylaminopurine) Seed-Priming Enhanced Growth, Antioxidant Accumulation and Anthocyanin Metabolism in Olive Sprouts

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Abstract: Given the importance of olive sprouts, it is crucial to explore their potential as an innovative source of bioactive and nutritive compounds through research. Here, we aimed at investigating the potential role of benzylaminopurine (BAP) in improving the tissue chemical composition and bioactivity of olive sprouts. To this end, seeds of two olive varieties (*Olea europaea* L. vr. Kroniaki and Coratina) were primed with BAP at 25 µM. A substantial enhancement was observed in biomass accumulation by 35% and 30% in Kroniaki and Coratina varieties, respectively. Likewise, the photosynthetic pigments (total chlorophyll, α- and β-carotene, lutein and β-cryptoxanthin) in both varieties were increased, mainly in Coratina. At primary metabolic level, BAP priming improved sprout lipid composition, particularly in Coratina variety. At antioxidant level, BAP priming improved lipid antioxidants (α-, β- and γ-tocopherols) and water-soluble antioxidants (phenols, flavonoids, ascorbate, glutathione and anthocyanins) in both olive varieties. At the anthocyanins level, their precursors (phenylalanine, cinnamic acid, coumaric acid and naringenin) and key biosynthetic enzyme activity (phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), coenzyme A ligase (4CL) and cinnamate 4-hydroxylase (C4H)) were improved in olive varieties, but to a greater extent in Coratina variety. Overall, the sprouts of BAP-primed olive seeds could potentially enhance their nutritional value, suggesting that the sprouts of BAP-primed olive seeds can be used as a food ingredient and additives.

Keywords: Seed priming; 6-benzylaminopurine; *Olea europeae* sprouts; phytochemicals; antioxidant activity

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1. Introduction

Olive (*Olea europeae*) is an evergreen tree or shrub native to Mediterranean Europe, Asia and Africa from ancient times. Olive is adapted to a wide agroecological zone, but nowadays there is increased degradation of olive groves [1]. The seeds of olives are a by-product of the pitted table olive industry [2]. To improve seed oil [3], several uses for this by-product have been researched, including fractionation, combustion, production of activated carbon, furfural, bio-oil, and resins, and extraction of phenolics and other phytochemicals [2]. Moreover, several studies have shown that sprouting seeds of several herbaceous species considerably improves their phytochemical content when compared to seeds. For example, sprouts are rich in bioactive compounds [4], thus they are considered a good source of minerals, vitamins, unsaturated fatty acids, antioxidants, essential amino acids and other nutrients that promote their biological function.

Other than the seed coat, endosperm and embryo itself, the stony endocarp of seeds is the major barrier to olive seed germination [1]. In this context, the endocarp and endosperm are responsible for 56% and 28% of the dormancy of olive seeds, respectively. In actuality, dormancy affects olive seeds and must be broken with specialized methods such as chemical scarification [5] or cold stratification [6]. To overcome physical barriers of seed dormancy and improve seed germination, seed priming is widely used. In this context, numerous priming techniques have successfully [7] improved seed germination and emergence of seedlings. Seed priming has been shown to induce biochemical changes in seeds, leading to improved germination rates, faster and more uniform emergence, and enhanced seedling vigor crops [8]. Priming can not only improve the seed germination, but it can also increase seedling quality and accumulation of bioactive metabolites [9]. Some of the common compounds used for seed priming include plant hormones (such as gibberellic acid or cytokinins), antioxidants, osmoprotectants (such as polyethylene glycol or mannitol) and beneficial microorganisms. For instance, priming of seeds with hormones such as 6-benzylaminopurine (BAP) can facilitate the processes of seed germination and seedling emergence [10]. It improved minerals and bioactive secondary metabolite accumulation [10]. Thus, olive sprouts can also be utilized as additives in various food products. They can be processed into powders, extracts or oils to impart their flavor and nutritional benefits to a wide range of food items.

While there are numerous studies available on the application of BAP to enhance plant bioactivity, as far as we know, the impact of BAP on enhancing the sprouting of olive seeds and the accumulation of bioactive metabolites has not been investigated yet. Only a few studies have shown that BAP priming improved sprouting and significantly enhanced the phytochemical content compared to their seeds. Hence, the aim of this work was to study the effect of hormonal (BAP) priming on enhancing the sprouting process by inducing accumulation of bioactive compounds in spouts of two olive varieties: Kroniaki and Coratina. Coratina is characterized by early production and its adaptability to different soils and climates. It is primarily used for olive oil production and is known for its robust flavor profile, as well as its high oil and polyphenol content [11]. Kroniaki is also known for high oil production and is rich in bioactive compounds such as polyphenols, terpenic acids and unsaturated fatty acids [12]. Thus, olive sprouts could be exploited more readily for food supplementation and pharmaceutics. We hypothesize that BAP will not only improve olive sprout growth, but will also improve its nutritive and health-promoting values of olive sprouts from two olive varieties. Moreover, our study introduces the sprouts of BAP-primed olive seeds as a food ingredient and additive.

2. Materials and Methods

2.1. Sprout Production

After collecting olive seeds of Kroniaki and Coratina varieties from whole stones, seeds were sterilized with sodium hypochlorite (2.7%) for 4 min. Then, seeds were treated for 30 days for stratification in the dark (at 15 °C) in plastic trays. The trays contained sterile peat wetted with distilled water and were moved to a growth cabinet under controlled

conditions (21/18 °C over a 16/8 h day/night photoperiod, 150 µmol PAR m^{-2} s^{-1}, with 60% humidity). BAP priming at a concentration of 25 µM was applied to seeds for 8 h [13,14]. Control seeds were hydro primed for 8 h. The BAP concentration of 25 µM was selected by preliminary sprouting experiments, where various concentrations (ranging from 0 to 50 µM) were assessed for their effects on the growth (fresh weight) and total antioxidant capacity (evaluated using the ferric reducing antioxidant power (FRAP) assay) of olive sprouts. The sprouts of olive seed primed with 25 µM BAP showed the highest growth and antioxidant capacity, therefore, it was selected. During seed germination, plants were watered twice per week and Hoagland nutrient solution was provided at the commencement of the experiment. After 10 days of germination, the fresh weight of the olive sprouts was measured and then sprout tissues were stored (−80 °C) for biochemical analyses. For each measurement, we utilized 6 biological replicates, with each replicate consisting of 15 sprouts.

2.2. Analysis of Pigment Profile

For pigment analysis, about 200 mg of fresh olive sprouts was ground in liquid nitrogen and homogenized for 30 s in 5 mL of 95.5% acetone using MagNALyser (Roche, Vilvoorde, Belgium) for 1 min, 7000 rpm. After homogenization and centrifugation for 20 min (14,000× *g*, 4 °C) [15], the sample's clear supernatant was filtered through an Acrodisc GHP filter with a 0.45 m pore size. With the help of high-performance liquid chromatography (HPLC, Shimadzu SIL10-ADVP), reversed phase at 4 °C, pigments such as chlorophylls, α-carotene, β-carotene, lutein and β-cryptoxanthin were analyzed. The pigments were fractionated on a silica-based C18 column. The mobile phase consisted of a mixture of acetonitrile, methanol and water (81:9:10, solvent A), along with methanol and ethyl acetate (68:32, solvent B). The mobile phase was injected at the rate of 1.0 mL/min at room temperature. Subsequently, pigments were identified with the assistance of a diode array detector, and Shimadzu Lab Solutions Lite software (Shimadzu UV-1800 series, software UV probe version 2.42, Tokyo, Japan) was employed for quantifying pigment concentrations.

2.3. Determination of Fatty Acid Levels

Fatty acid levels in olive sprouts were quantified by GC/MS (MSD 5975-mass spectrometer) [16]. Methanol (100%) was applied to extract fatty acids from 250 mg of fresh olive sprouts at 24 °C. The internal standards (nonadecanoic acids) were added during extractions. GC-MS analysis was conducted using a Hewlett Packard 6890 coupled with an MSD 5975. Fatty acids were separated on an HP-5 MS column with a length of 30 m, an internal diameter of 0.32 mm and a film thickness of 0.25 µm. To identify the fatty acids, both the NIST 05 database and the Golm Metabolome Database, accessible at http://gmd.mpimp-golm.mpg.de (accessed on 5 March 2023), were employed. The concentration of each molecule was calculated by comparing the peak area of each chemical to a calibration curve of the pertinent standard.

2.4. Quantification of Lipid Antioxidant Metabolites

Tocopherols were quantified by using HPLC (normal phase conditions, Particle Pac 5 µm column material) [17]. Tocopherols were extracted from 0.2 g of fresh sprouts using 5 mL of hexane, followed by centrifugation at 13,500× *g* for 27 min. The resulting extracts were then dehydrated using a CentriVap concentrator (Labconco in Kansas, MO, USA), and subsequently reconstituted in hexane. The quantification of tocopherols was performed through HPLC using a Shimadzu instrument located in Hertogenbosch, The Netherlands, under normal phase conditions. Separation was achieved using a Particle Pac 5 mm column with dimensions of 220 mm in length and 4.5 mm in inner diameter. Dimethyl tocol (DMT) was employed as an internal standard at 5 ppm.

2.5. Quantification of Water-Soluble Antioxidant Metabolites

The total phenols and flavonoids were also extracted from 100 mg of fresh sprouts in 80% ethanol to assess their total concentration. The Folin–Ciocalteu assay and Al (III)-flavonoid complex colorimetric method were used to quantify the phenolic content and flavonoid content, respectively. Gallic acid was employed as a standard for quantifying total phenolic content, and the results were reported as µmol GAE/g FW. As for flavonoids, the color produced was measured at 417 nm, and the findings were expressed as µmol QE/g FW.

For ascorbic acid (ASC) and glutathione (GSH) measurements, fresh sprouts were extracted in meta-phosphoric acid (6%, *w/v*). These antioxidants were measured by a reversed phase of HPLC analysis (Shimadzu, Hertogenbosch, The Netherlands) after separation on a C18-A column (Polaris C18-A 100 mm length × 4.6 mm internal diameter) using diode array detector (DAD) [18]. The components were quantified utilizing a bespoke electrochemical detector and the total GSH and ASC measured after reduction with dithiothreitol (0.04 M).

2.6. Determination of Anthocyanin Content, Percussors and the Activity of Related Biosynthetic Enzymes

Anthocyanins were obtained through extraction using methanol:HCl in a 99:1 (*v/v*). Following this, the homogenate of fresh sprouts was placed in darkness and incubated at 24 °C for 22 h, after which it underwent centrifugation at 3500× *g* for 15 min. To determine the anthocyanin content of the extract, its absorbance at 545 nm was measured, and the [19] phenylalanine ammonia-lyase (PAL) extraction was performed [19]. Sample preparation involved grinding one gram of fresh sprout tissue with a pestle and mortar using liquid N, followed by the addition of 12 mL of acetone. This mixture was then placed at −20 °C for 12 min, filtered, washed twice with cold acetone, and dried at 24 °C. The activity of PAL was monitored by measuring the absorbance of trans-cinnamic acid at 290 nm. To assay 4-coumarate: coenzyme A ligase (4CL) activity, the increase in coumarate was measured at 333 nm. Fresh samples were extracted in Tris-HCL buffer (pH = 8.9), and the activity was monitored by measuring the absorbance of the product, 4-hydroxy-trans-cinnamic acid. Protein content was determined using the Folin–Lowry method, where 0.2 g of frozen olive sprouts was homogenized in a chloroform/methanol (2:1, *v/v*) solution and centrifuged at 3000× *g* for 15 min to measure the total protein content [19].

2.7. Total Antioxidant Capacity

The total antioxidant capacity was extracted from 100 mg olive sprouts in 80% ethanol solution by shaking. After centrifugation (14,000× *g*, 4 ºC, 25 min). The total antioxidant capacity was performed by using FRAP assay using reaction buffer containing 0.3 M acetate buffer (pH 3.6), 0.01 mM TPTZ in HCl (0.04 mM) and 0.02 M $FeCl_3$. Trolox was applied as a standard [20,21]. The values were expressed as µmol Trolox equivalents/g of plant extract using the standard curve established previously. Extract supernatants were also mixed with DPPH reagent to measure total antioxidant capacity as DPPH at 517 nm using the spectrometric method [22].

2.8. Statistical Analyses

One-way analysis (ANOVA) was carried out using SPSS v25.0 (SPSS, Inc., Chicago, IL, USA). Tukey's test ($p < 0.05$) was used. Data normality was checked by using Levene's test. Data were also checked by a Bartlett's test for equal variances. Six biological replicates (n = 6) were conducted.

3. Results

3.1. BAP Improved Biomass Accumulation in Kroniaki and Coratina Varieties

The effect of BAP treatment on the biomass accumulation of Kroniaki variety and Coratina variety sprouts (Figure 1). The fresh (FW) and dry weight (DW) of sprouts were

significantly increased by BAP priming. The highest increase was recorded for the sprouts of BAP-primed seeds of Kroniaki variety (35%) as compared to the sprouts of BAP-primed olive seeds of Coratina variety (30%). Overall, this indicated that BAP priming equally increased the growth of the two sprout varieties.

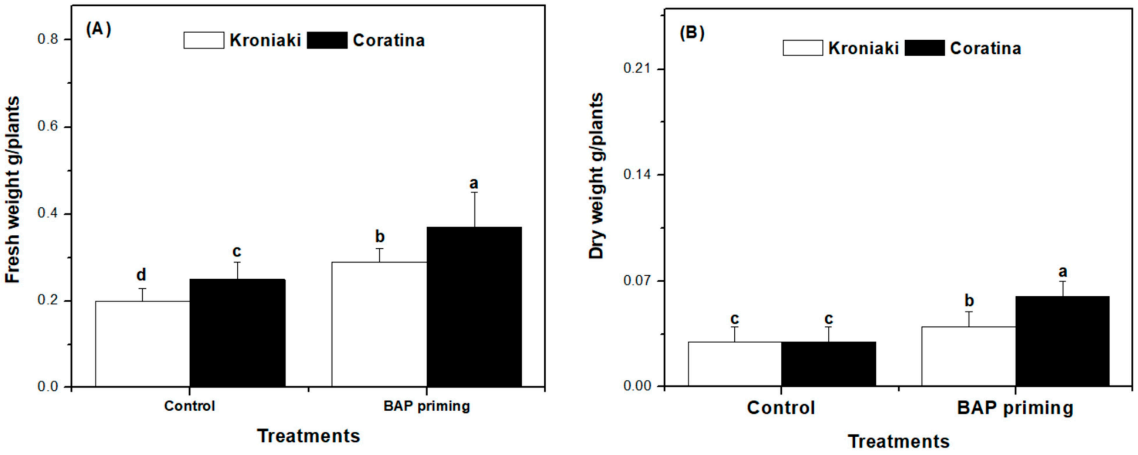

Figure 1. Effect of BAP priming (25 µM, 8 h) on growth parameters including fresh weight (**A**) and dry weight (**B**) of the two varieties of 10-day-old olive sprouts (Kroniaki and Coratina). Data are represented by means ± standard errors. Different small letters (a, b, c and d) above bars indicate significant differences in the two varieties between the means of control and BAP treatments ($p < 0.05$).

3.2. BAP Differentially Increased the Photosynthetic Pigments in Kroniaki and Coratina Varieties

Depending on olive varieties, differential increases in the pigment's levels were observed (Figure 2). There were significant ($p < 0.05$) increases in total chlorophyll, α- and β-carotene, lutein and β-cryptoxanthin by 33%, 33%, 26%, 35% and 36%, respectively, in the sprouts of BAP-primed olive seeds of Coratina variety, which exhibited significant increases in pigment levels but to less extent as compared to the sprouts of BAP-primed olive seeds of Kroniaki variety.

3.3. BAP Increased Fatty Acids and Lipid Antioxidant Accumulation in Kroniaki and Coratina Varieties

Saturated and unsaturated fatty acid compositions of the sprouts of BAP-primed olive seeds were investigated (Table 1). The obtained results showed that Coratina variety showed higher fatty acid levels than Kroniaki variety under both control and BAP treatment conditions. When exposed to BAP priming, fatty acid levels exhibited distinct behavior. Compared to the control, the BAP priming treatment resulted in a significant increase in saturated fatty acids (stearic (C18:0), arachidic (C20:0), docosanoic (C22:0), tricosanoic (C23:0), pentacosanoic (C25:0)) and unsaturated fatty acids (palmitoleic (C16:1), heptadecenoic (C17:1), oleic (C18:1) and linoleic (C18:2)) in the sprouts of the varieties. On the other hand, a significant decrease in arachidic acid (C20:0) and eicosenoic (C20:1) was observed in the sprouts of BAP-primed olive seeds of the two olive varieties (Table 1).

The lipid antioxidants (tocopherols) contribute to preventing lipid oxidation by scavenging free radicals and interacting with singlet oxygen. BAP priming also significantly increased the content of individual α-, β-, γ- as well as the total tocopherol content in sprouts of both olive varieties (Table 2). The highest increase was recorded for Coratina variety by 33%, 32%, 16% and 33% in α-, β-, γ- and total tocopherol levels compared to control, respectively. Similarly, the sprouts of BAP-primed olive seeds of Kroniaki variety exhibited significant increases in tocopherol levels but to less extent than the Coratina variety.

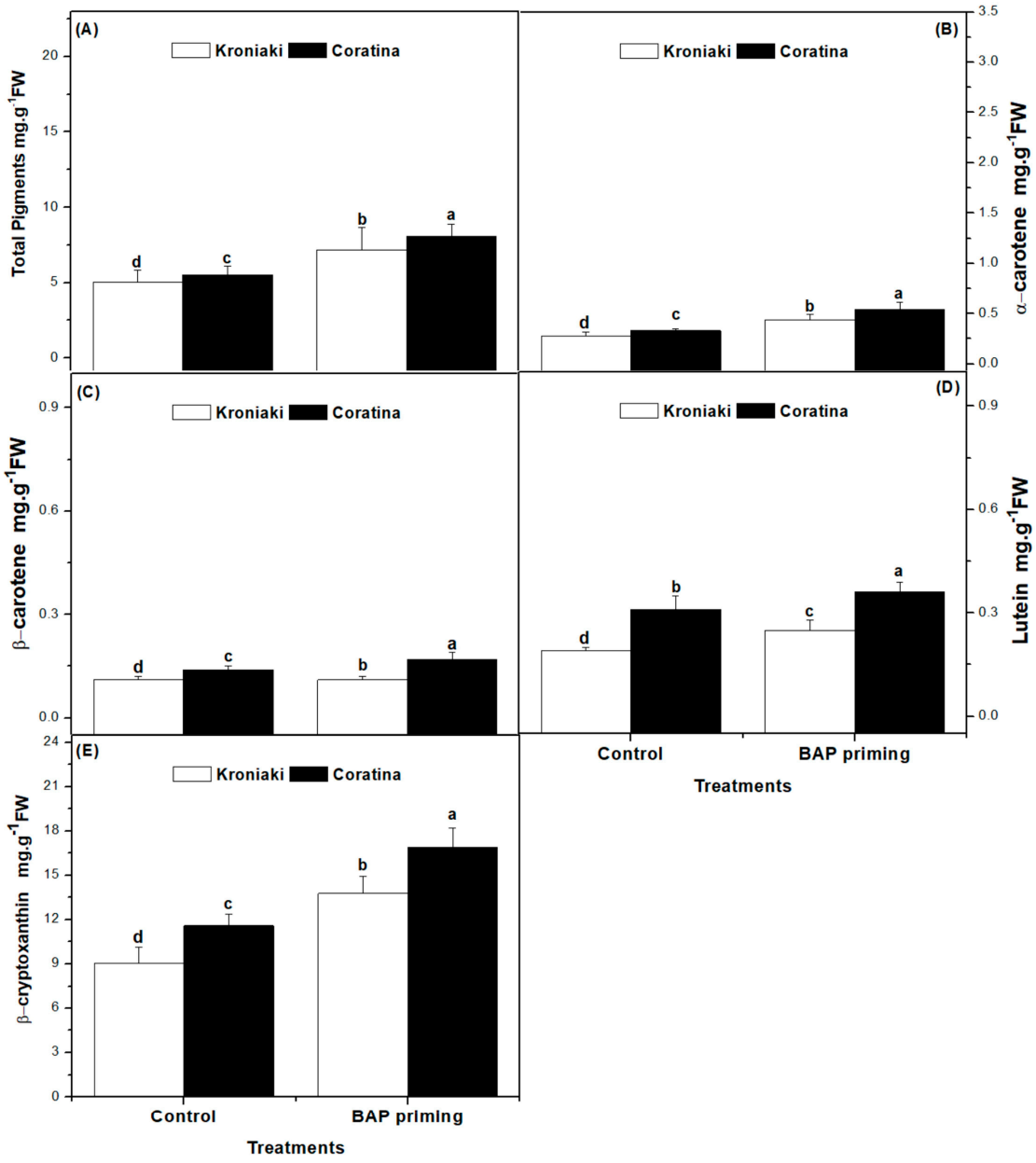

Figure 2. Effect of BAP priming (25 µM, 8 h) on leaf pigments including (**A**) total pigments, (**B**) α-carotene, (**C**) β-carotene, (**D**) Lutein, and (**E**) β-cryptoxanthin levels of the two varieties of 10-day-old olive sprouts (Kroniaki and Coratina). Data are represented by means ± standard errors. Different small letters (a, b, c and d) above bars indicate significant differences in the two varieties between the means of control and BAP treatments ($p < 0.05$).

3.4. The Sprouts of BAP-Primed Olive Seeds Accumulated High Level of Water-Soluble Antioxidants

A plant extracts as an antioxidant are significantly reflected by the levels of water-soluble phenolics and flavonoids. The current findings showed that phenolic components in sprouts of both olive varieties were altered in the sprouts of BAP-primed olive seeds (Figure 3). It significantly ($p < 0.05$) increased total phenolic and flavonoid contents. For instance, total phenolic and flavonoid contents were increased in the sprouts of Kroniaki variety by 31% and 35%, respectively. Additionally, a significant ($p < 0.05$) increase in the total phenolic and flavonoid contents was observed in Coratina variety, where they were increased by about 32% and 31%, respectively (Figure 3).

Table 1. Effect of 6-benzylaminopurine (BAP) priming (25 µM, 8 h) on fatty acid profile of the two varieties of 10-day-old olive sprouts (Kroniaki and Coratina).

Varieties	**Kroniaki**		**Coratina**	
mg.g^{-1} FW	**Control**	**BAP**	**Control**	**BAP**
Myristic (C14:0)	1.08 ± 0.07 c	1.64 ± 0.04 b	1.69 ± 0.22 b	2.06 ± 0.04 a
Palmitic (C16:0)	15.13 ± 5.11 b	27.23 ± 5.02 a	17.28 ± 8.26 b	25.92 ± 4.98 a
Heptadecanoic (C17:0)	0.07 ± 0.01 b	0.07 ± 0.01 b	0.14 ± 0.01 a	0.14 ± 0.01 a
Stearic (C18:0)	2.97 ± 0.05 b	4.3 ± 0.32 a	3.59 ± 0.52 a	6.00 ± 0.83 a
Arachidic (C20:0)	1.56 ± 0.29 b	1.71 ± 0.12 ab	20.4 ± 0.3 ab	2.24 ± 0.13 a
Docosanoic (C22:0)	0.86 ± 0.06 b	1.15 ± 0.08 a	0.82 ± 0.03 b	1.47 ± 0.19a
Tricosanoic (C23:0)	0.06 ± 0.02 b	0.04 ± 0.01 b	0.12 ± 0.01 a	0.04 ± 0.01 b
Pentacosanoic (C25:0)	0.01 ± 0.00 a	0.00 ± 0.00 b	0.01 ± 0.00 a	0.01 ± 0.00 a
Palmitoleic (C16:1)	0.13 ± 0.01 b	0.12 ± 0.03 b	0.26 ± 0.07 a	0.26 ± 0.07 a
Heptadecenoic (C17:1)	0.34 ± 0.12 b	0.44 ± 0.10 a	0.29 ± 0.03 b	0.48 ± 0.19 a
Oleic (C18:1)	68.2 ± 2 c	101.7 ± 4 b	82.8 ± 4 b	128.2 ± 19 a
Linoleic (C18:2)	11.7 ± 1.38 b	19.04 ± 0.51 ab	11.33 ± 1.15 b	19.18 ± 1.24 a
Linolenic (C18:3 ω−3)	1.13 ± 0.13 a	0.05 ± 0.02 c	1.17 ± 0.26 a	0.77 ± 0.09 b
Arachidonic (C20:4)	ND	ND	1.11 ± 0.44 b	2.01 ± 0.32 a
Eicosenoic (C20:1)	0.51 ± 0.05 a	0.22 ± 0.05 b	ND	ND

Data are represented by means ± standard errors. Different small letters (a, b, c) in the same row indicate significant differences between the control and BAP treatment in the two varieties ($p < 0.05$). Data were statistically analyzed by one-way ANOVA followed by Tukey's post hoc test.

Table 2. Effect of 6-benzylaminopurine (BAP) priming (25 µM, 8 h) on alpha (α), beta (β) and gamma (γ) tocopherol levels of the two varieties of 10-day-old olive sprouts (Kroniaki and Coratina).

Varieties	**Kroniaki**		**Coratina**	
mg.g^{-1} FW	**Control**	**BAP**	**Control**	**BAP**
α-tocopherol	1.93 ± 0.44 b	2.87 ± 0.21 a	2.32 ± 0.15 b	3.39 ± 0.41 a
β-tocopherol	0.29 ± 0.02 b	0.49 ± 0.04 b	0.37 ± 0.02 b	0.60 ± 0.04 a
γ-tocopherol	0.09 ± 0.02 c	0.08 ± 0.01 c	0.10 ± 0.00 b	0.15 ± 0.04 a
Total tocopherols	1.89 ± 0.20 c	3.42 ± 0.25 a	2.47 ± 0.27 c	3.68 ± 0.38 a

Data are represented by means ± standard errors. Different small letters (a, b, c) in the same row indicate significant differences between the control and BAP treatment of the two varieties ($p < 0.05$). Data were statistically analyzed by one-way ANOVA followed by Tukey's post hoc test.

Similar to the increases in phenolic and flavonoid levels, ASC and GSH accumulations were also observed. Likewise, a significant increase in total ASC and GSH was recorded in both varieties of olive sprouts ($p < 0.05$) (Figure 4). This indicated that BAP priming improved the redox status in the sprouts of both olive varieties.

3.5. Anthocyanin Metabolism Induction in the Sprouts of BAP-Primed Olive Seeds

Anthocyanins are well known as antioxidants and nutritive metabolites. BAP priming significantly improved anthocyanin accumulation in the sprouts of both olive varieties, but to a greater extent in the Coratina variety (Table 3). BAP priming treatments led to increased levels of anthocyanin biosynthetic precursors (phenylalanine, coumaric, cinnamic acid and naringenin). At anthocyanin metabolism, the anthocyanin percussor (L-phenylalanine) levels were significantly increased ($p < 0.05$) in the sprouts of both olive varieties. Phenylalanine increased in the sprouts of BAP-primed olive seeds of both olive varieties by 88% in Kroniaki variety and by 52% in Coratina variety compared to control (Table 3). Cinnamic acid was considerably ($p < 0.05$) greater in the sprouts of BAP-primed olive seeds of Kroniaki variety and Coratina variety (Table 3). Regarding biosynthetic enzymes, we also measured the enzymatic activities that are involved in anthocyanin biosynthesis to better explain these results. PAL enzyme activity was significantly ($p < 0.05$) increased in sprouts of both olive varieties after BAP priming treatment. However, this effect of BAP priming on PAL enzyme had a substantial ($p < 0.05$) impact on the Coratina variety. Sprouts

of both olive varieties showed considerable ($p < 0.05$) increase in the 4CL enzymatic activity. On the other hand, the sprouts of BAP-primed olive seeds of Coratina variety showed a significant ($p < 0.05$) increase in C4H enzymatic activity.

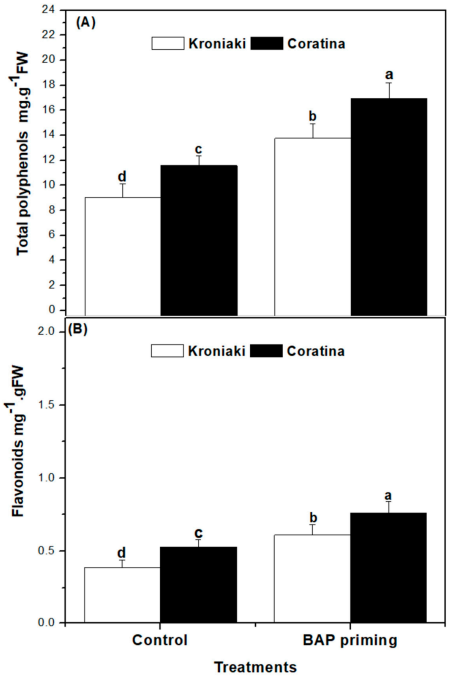

Figure 3. Effect of BAP priming (25 µM, 8 h) on total polyphenol (**A**) and flavonoid (**B**) contents of the two varieties of 10-day-old olive sprouts (Kroniaki and Coratina). Data are represented by means ± standard errors. Different small letters (a, b, c and d) above bars indicate significant differences in the two varieties between the means of control and BAP treatments ($p < 0.05$). Data were statistically analyzed by one-way ANOVA followed by Tukey's post hoc test.

Table 3. Effect of 6-benzylaminopurine (BAP) priming (25 µM, 8 h) on anthocyanin metabolism of the two varieties of 10-day-old olive sprouts (Kroniaki and Coratina).

Varieties	Kroniaki		Coratina	
	Control	**BAP**	**Control**	**BAP**
Metabolite level (mg. g^{-1} FW)				
Anthocyanin	1.04 ± 0.39 c	2.50 ± 0.28 c	3.29 ± 1.23 b	6.19 ± 0.13 a
Phenylalanine	1.04 ± 0.12 c	1.68 ± 0.16 a	1.30 ± 0.17 b	1.96 ± 0.24 a
Cinnamic acid	6.04 ± 0.51 b	9.59 ± 1.23 a	8.12 ± 1.05 b	11.44 ± 0.59 a
Coumaric acid	1.89 ± 0.23 b	1.71 ± 0.17 b	3.74 ± 0.84 a	3.63 ± 0.47 a
Naringenin	6.41 ± 0.86 c	5.38 ± 0.8 b	9.9 ± 0.54 ab	9.32 ± 0.41 a
Enzyme activity (µmol. mg^{-1} protein. g^{-1} FW)				
Phenylalanine ammonia lyase	2.81 ± 0.22 b	1.97 ± 0.5 b	2.70 ± 0.41 a	3.22 ± 0.74 a
Chalcone synthase	0.83 ± 0.14 c	1.09 ± 0.14 a	1.04 ± 0.14 b	2.01 ± 0.22 c
Cinnamate 4-hydroxylase	2.75 ± 0.37 b	4.32 ± 0.25 b	2.10 ± 0.11 b	1.23 ± 0.31 a
Coenzyme A ligase	0.40 ± 0.07 b	0.37 ± 0.08 a	1.06 ± 0.06 a	1.48 ± 0.19 b

Data are represented by means ± standard errors. Different small letters (a, b, c) in the same row indicate significant differences between the control and BAP treatment in the two varieties ($p < 0.05$). Data were statistically analyzed by one-way ANOVA followed by Tukey's post hoc test.

3.6. BAP Priming Effect on Antioxidant Capacity of Olive Sprouts

The current investigation also revealed considerable increases in the total antioxidant capacities of olive sprouts, as indicated by DPPH and FRAP scavenging potentials (Figure 5). BAP priming enhanced FRAP (35%, 30% in Kroniaki and Coratina, respectively) and DPPH

(38%, 42% in Kroniaki and Coratina, respectively) radical scavenging activities as compared to control.

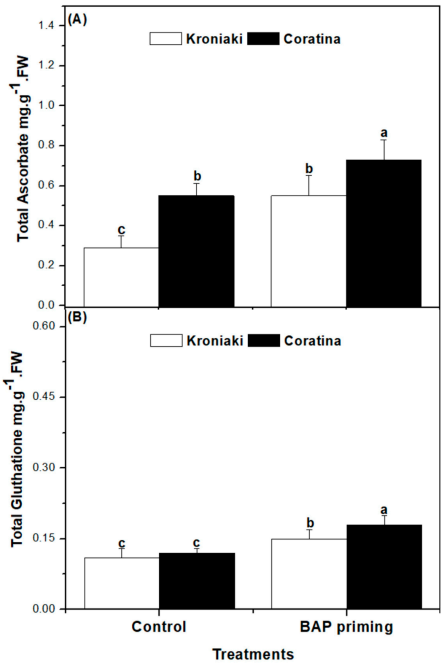

Figure 4. Effect of BAP priming (25 µM, 8 h) on total ascorbate (ASC) (**A**) and glutathione (GSH) (**B**) content of the two varieties of 10-day-old olive sprouts (Kroniaki and Coratina). Data are represented by means ± standard errors. Different small letters (a, b and c) above bars indicate significant differences between the control and BAP treatment in the two varieties ($p < 0.05$). Data were statistically analyzed by one-way ANOVA followed by Tukey's post hoc test.

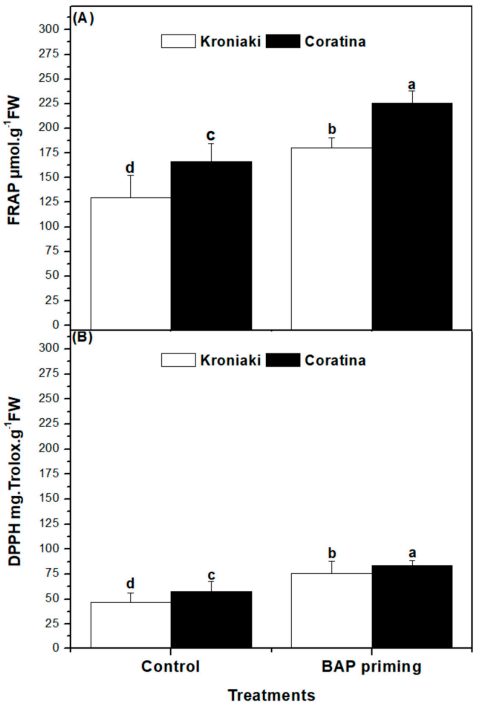

Figure 5. Effect of BAP priming (25 µM, 8 h) on total antioxidant capacity including FRAP (**A**) and DPPH (**B**) content of the two varieties of 10-day-old olive sprouts (Kroniaki and Coratina). Data are represented by means ± standard errors. Different small letters (a, b, c and d) above bars indicate significant differences between the control and BAP treatment in the two varieties ($p < 0.05$).

4. Discussion

4.1. BAP Priming Improved Sprouting Growth

Seed priming triggered the growth and activation of metabolic processes inherent to seeds. For instance, it enhanced seed germination, improved seedling vigor and provided better protection against environmental stresses [8]. Seeds priming with phytohormones is a widely practiced technique employed to improve seed germination and metabolic processes. In this regard, BAP is a synthetic cytokinin plant growth regulator commonly used in plant tissue culture and some agricultural practices. Here, BAP priming improved biomass accumulation in olive sprouts. In line with our results, seed priming with BAP improved seed germination [8] and growth parameters of linseed sprouts [20]. According to our results, BAP priming-induced growth improvement was dependent on olive variety. For instance, compared to Coratina variety, Kroniaki variety showed higher biomass accumulation. In this regard, research studies on *Medicago* sprouts showed variations in emergence and growth among different plant varieties [21]. Furthermore, increased leaf pigments can explain the improvement in growth metrics of the sprouts of BAP-primed olive seeds. Here, the BAP priming improved the total chlorophyll as well as carotenoids, including α-carotene and β-carotene and xanthophyll, like lutein and β-cryptoxanthin. Similar to BAP treatment, cytokinin application improved photosynthetic pigments [22,23]. Furthermore, it is widely recognized that the external application of BAP elevates cytokinin levels, subsequently fostering germination [24,25].

4.2. BAP Priming Improved the Nutritional Values of Olive Sprouts

The subsequent mobilization of the major seed storage reserves is associated with improved sprout germination [24]. Therefore, it is important to delve into the underlying biochemical bases that drive these processes, including the activation of metabolic activities [25]. In this context, it is worth noting that the metabolic processes, such as bioactive metabolite accumulation induced by BAP, may have a direct correlation with the enhancement of the nutritive values of olive sprouts. In the present investigation, BAP priming improved the accumulation of primary and secondary metabolites and induced the antioxidant activity of olive variety sprouts. As the primary metabolites, olive oils encompass increased levels of several unsaturated fatty acids, which indicates enhanced biological benefits. This qualitative pattern showed resemblance with that found in olive seeds [8]. It is also likely that hormones boosted overall capacity of the cells for biosynthesis of active primary metabolites [26]. BAP priming enhanced the accumulation of six fatty acids in the sprouts of both olive varieties. Interestingly, oleic acid is the primary monounsaturated fatty acid generated and exported by plastids, the predominance of oleic acid in the sprouts of both olive varieties [27,28]. Additionally, our findings demonstrated that BAP priming increased the production of linolenic acid. The increased oleic acid concentration in sprouts as a result of BAP priming suggested an increase in diene and triene fatty acid biosynthesis [29]. This was most likely caused by cytokinin's capacity to promote photosynthesis-induced primary metabolism [30].

Olive oil is more resistant to heat oxidation during frying in addition to having antioxidant potential in biological systems [31]. For instance, oil rich in vitamin E, a fat-soluble vitamin, supra-nutritional vitamin E levels have been shown to have positive benefits on conditions like Alzheimer's, Parkinson's, cancer and chronic inflammation [8]. Thus, increased tocopherol levels suggest a high biological activity [31,32]. Among tocopherols, α-tocopherol is the most prevalent vitamin [33]. It has an antioxidant role that protects cell membranes from lipid peroxidation and scavenges reactive oxygen species, protecting tissues from oxidative damage as a result. In our study, α- and γ-tocopherols were the most prevalent tocopherols in olive sprouts. BAP priming enhanced the accumulation of total tocopherol, mainly in Coratina variety, and improved the α-, β-, and γ-tocopherol forms in both olive varieties. Similar results were observed, and this variation in the content of specific α, β, and γ-tocopherols was also found to be dependent on the type of sprouts studied [34].

In addition, aside from the bioactive functions of lipid antioxidant tocopherols, the notably high levels of carotenoid and xanthophyll pigments (water-soluble antioxidants) also indicate an enhanced biological value of the sprouts of BAP-primed olive seeds. Therefore, we considered their potential roles in disease prevention, as assessed in previous studies [33]. For instance, lutein has potent anti-inflammatory and antioxidant effects and is highly effective against liver tumors [35]. As water-soluble antioxidants, the total phenol and flavonoid contents were improved by BAP priming in both varieties. In the previous study by Okla et al. [36], BAP priming increased the accumulation of water-soluble antioxidants [37]. Increased levels of such antioxidant metabolites (phenolic, flavonoids, ASC, GSH, anthocyanin) can significantly help in free radical scavenging.

It is important to note that the specific effects of seed priming on secondary metabolites and the overall nutritive value of olive sprouts can vary depending on various factors, including the plant varieties [26]. In this context, flavonoids and ASC concentration increased to a greater extent in the Coratina variety compared to the Kroniaki variety. Furthermore, the heightened antioxidant activity observed in the Kroniaki variety appeared to be attributed to the accumulation of anthocyanins in response to BAP priming. On the other hand, Coratina variety of sprouts showed the greatest increase in anthocyanin content, indicating that BAP was a positive regulator of the pathway involved in producing anthocyanins. Regarding anthocyanin synthesis in sprouts, it depends on the specific plant species and cultivar, while some sprouts exhibit anthocyanin synthesis, such as broccoli sprouts or cabbage sprouts. Moreover, it is important to note that the anthocyanin content in sprouts can vary based on growing conditions, harvest time, and plant genetics. These factors are mediated via a variety of regulatory elements that function at the transcriptional level [38]. Numerous plants, including the carrot, artichoke, *Haplopappus gracilis*, garden balsam, rose and grape, have shown that BAP stimulates the accumulation of anthocyanins [39]. Hence, BAP-induced stimulation of anthocyanin biosynthesis could serve as a valuable tool in exploring the cytokinin action mechanism. This is facilitated by the extensive knowledge available regarding the molecular biology and genetics associated with anthocyanin biosynthesis [40]. Overall, when seeds germinate, they undergo biochemical changes that result in increased nutrient content compared to the mature plant. Therefore, sprouts are considered a nutrient-dense food and can be a valuable addition to a healthy diet.

5. Conclusions

Olive sprouts can be used as ingredients in various food products. When they are used as ingredients, they contribute to the overall nutritional composition. BAP priming further improved the nutritive value of olive sprouts. BAP priming increased lipid- and water-soluble antioxidants and stimulated anthocyanin biosynthesis. This influence of BAP priming displayed unique responses depending on the variety. The elevated levels of these bioactive compounds may hold potential benefits in the prevention of various human diseases.

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